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Quantitative Development of Adipose Tissue in Foetal Sheep

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Abstract

The development of lipid stores in foetal sheep was examined using quantitative dissection and chloroform extraction.

The accumulation of extractable lipid commenced prior to the 50th day of foetal age; growth of dissectible perirenal–abdominal adipose tissue and other non-subcutaneous depots (all brown fat) commenced at about day 70, while growth of the subcutaneous adipose tissue (white fat) commenced 2–3 weeks later.

The subcutaneous fat regressed markedly from about day 115 and had virtually disappeared by full term. There were also consistent trends (not all statistically significant) in various indices of lipid status indicating that the rate of lipid accumulation was declining near term; these indices include percentage of lipid in adipose tissue, in the body as a whole and in skeletal muscle, and the rate of growth of the perirenal–abdominal adipose depot.

The involution of subcutaneous adipose tissue appeared to be hastened by maternal nutritional restriction, but was not prevented by feeding the ewes *ad libitum* on a high-energy diet. There were also consistently lower values of the indices of lipid status in foetuses from nutritionally restricted ewes than in foetuses from ewes fed *ad libitum*.

The early regression of the white subcutaneous adipose tissue suggests that the normal foetal lamb may be in a state of undernutrition during the last 5 or so weeks of pregnancy.

Hypophysectomized foetuses, full term or post-term, were comparatively obese, with a thick layer of subcutaneous fat, a high proportion of other adipose tissue depots in the body, and a high content of chloroform-extractable lipid.

Introduction

Lipid is of importance to the metabolism of newborn lambs. It is the substrate used by brown adipose tissue for non-shivering thermogenesis (Alexander and Williams 1968; Smith and Horwitz 1969), and it is the major source of energy in unsuckled lambs (Alexander 1962). Much of the lipid reserve in newborn lambs lies in the adipose tissue, but normal lambs of various breeds (Merino, Corriedale, Border Leicester, Dorset Horn, Border Leicester × Merino) possess little or no subcutaneous fat (Alexander and Bell 1975) in contrast to hypophysectomized foetuses which possess substantial subcutaneous depots (Liggins and Kennedy 1968). In normal lambs almost all of the adipose tissue, amounting to about 1·5% of body weight, is of the brown variety (Gemmell *et al.* 1972; Alexander and Bell 1975). The non-adipose lipid reserves appear to be as large as those in the adipose tissue (Alexander and Bell 1975), but their location has not been identified.

Despite the importance of lipid to newborn lambs, there has been no detailed report of the prenatal development of lipid stores in sheep. This paper presents quantitative data from a study on this topic. The investigation was primarily designed as an ultrastructural examination of the nature and development of adipose tissue in normal and hypophysectomized sheep foetuses (Gemmell and Alexander 1978), an examination that showed that any subcutaneous adipose tissue was white fat.

During the early phases of this 2-year study foetuses with an obvious covering of subcutaneous fat were unexpectedly encountered, contrasting with full-term newborn lambs examined previously (Alexander and Bell 1975) in which no more than traces of subcutaneous fat were observed. It seemed that this difference could have been nutritional in origin, since maternal nutrition is known to influence lipid reserves in newborn lambs (Wallace 1948; Pálsson and Vergés 1952; Alexander 1962, 1974). The study was therefore expanded to include foetuses from ewes on widely different feeding regimes.

Materials and Methods

Outline of Study

Lipid contents and sizes of adipose depots were examined in three series of foetuses delivered by Caesarian section. In the *age series* Merino foetuses were collected at ages ranging from 40 days to normal full term (150 days). In the *nutrition series* some ewes were fed, from the 90th day of pregnancy, on large amounts of a high-energy diet while the other ewes were fed restricted amounts of the medium-energy diet; the foetuses were collected when 123–127 or 146–148 days old. In the *hypophysectomy series* foetuses were collected when 148–150 or 164–166 days old, the removal of the pituitary between days 95 and 115 having prevented parturition in accordance with the findings of Liggins and Kennedy (1968).

Animals

Fine-woolled Merino ewes that had lambed several times previously were mated with rams of similar breeding. Each ewe was exposed to fertile rams for a single oestrus only, so that there could be no subsequent doubt about the age of the foetuses. Ewes that were subsequently detected in oestrus by vasectomized rams were rejected.

Table 1. Numbers of single and twin lambs in each series

	Foetal age (days)	No. of ewes	No. of lambs[A] Singles	Twins
Age series	40–150	35	24	22
Nutrition series[B]				
Restricted	123–127	6	4	4
	146	4	4	—
Ad libitum	125–126	3	2	2
	146–148	6	6	—
Hypophysectomy series	148–150	4	4	—
	164–166	4	4	—

[A] The variables studied were not examined in all lambs.
[B] The original object was to have six ewes in each treatment, but due to various mishaps this was not achieved.

Ewes were kept on pasture for the first 12 weeks of pregnancy. Ewes in the hypophysectomy series were then transferred to group pens in an animal house, and ewes of the age and nutrition series to single pens, until the foetus was taken at Caesarian section or, in one instance, until the lamb was born. The numbers of animals and the incidence of twins in the three series are shown in Table 1.

Ewes of the age series were fed a ration consisting of 60% chopped lucerne hay and 40% oat grain at the daily rate of 20 g/kg body weight. Ewes in the hypophysectomy series were group-fed the same diet at the same mean rate (20 g/kg). In the nutrition series some animals, termed the 'restricted' ewes, were fed the same diet at the daily rate of only 10 g/kg, while the remaining ewes, termed the '*ad libitum*' ewes, received a mixture of chopped lucerne hay (50%), crushed corn (30%), soybean meal (15%) and formaldehyde-treated casein (5%) at the daily rate of 35 g/kg; this ration was close to *ad libitum* as residues were sometimes left by some ewes. Rations were calculated from the body weight 12 weeks after mating; the weights ranged from 31 to 47 kg but most were close to 40 kg.

Foetal Hypophysectomy

Electrocoagulation of the foetal pituitary was carried out by the technique of Liggins *et al.* (1967)—an insulated probe was passed through the uterine wall, into the frontal bone in the midline of the skull and thence into the hypophyseal fossa.

Treatment and Dissection of Foetuses

Lambs that breathed spontaneously after delivery were killed with pentobarbitone-sodium anaesthetic. Following sampling for ultrastructural study the animals were dried with towels, weighed, and stored in plastic bags at 4°C until dissected during the next 1–3 days. The major discrete adipose tissue depot (perirenal–abdominal) and three minor depots (popliteal, postorbital and pericardial) (Alexander and Bell 1975) were dissected out as completely as possible and weighed. The approximate thickness and distribution of any subcutaneous adipose tissue were recorded and dissection of this tissue on the trunk was attempted in most animals. The biceps femoris, semitendinosus and semimembranosus skeletal muscles from one hind leg were also collected from each lamb. The muscles were separated and trimmed of all visible adipose tissue. The liver was also collected and weighed.

Determination of Lipid Content

The pooled samples of dissected adipose tissue, the muscles of the hind leg, the liver, and the remainder of the lamb were each minced, mixed thoroughly and sampled; 12 g of mince, or the whole tissue if it was less than 12 g, was ground with anhydrous sodium sulphate, and the fat (neutral lipid) extracted for 3 h with chloroform, dried overnight at 100°C and weighed (Snook 1939).

Results

Weight Changes of Ewes

Ewes of the *age series* tended to gain weight slowly while on controlled feeding from the 12th week of pregnancy (mean gain 0·06 kg/day). In the *nutrition series* all restricted ewes lost weight (mean loss 0·11 kg/day), while all ewes on the high-energy diet gained weight rapidly (mean gain 0·22 kg/day). Weights of ewes in the *hypophysectomy series* were not recorded.

Foetal Weight

In the *age series* foetal growth followed an exponential type curve that appeared independent of whether lambs were singles or twins, at least in foetuses of 110 days or younger; in older foetuses variability was high (3·0–5·0 kg at about 145 days) and there were few twins (Fig. 1).

The weights of foetuses from ewes on the *ad libitum* diet lay close to, or above, the mean line for the age series, while those of foetuses from restricted ewes almost all lay below the line (Fig. 1). With single lambs the mean weights in these two groups of the *nutrition series* were significantly different at 146–148 days (Table 2) ($P < 0{\cdot}01$); the difference was also significant with twins ($P \simeq 0{\cdot}05$) at 125–127 days. Increases with age were clear and significant ($P < 0{\cdot}01$) (Table 2).

The weights of the *hypophysectomized* foetuses were all below the mean line for the age series, extrapolated to 165 days (Fig. 1).

Weight of Adipose Tissue

Subcutaneous depot

No dissectible subcutaneous adipose tissue was found in *age series* foetuses younger than 89 days or lighter than 0·4 kg, and the content in older and heavier foetuses, as indicated by dissection on the trunk region, was widely variable (Figs 2 and 3). The depot was rarely more than 1 mm thick. Relative to body weight the tissue appeared to make its maximum growth between 90 and 110 days and all 13 foetuses examined between 89 and 123 days inclusive had some dissectible sub-

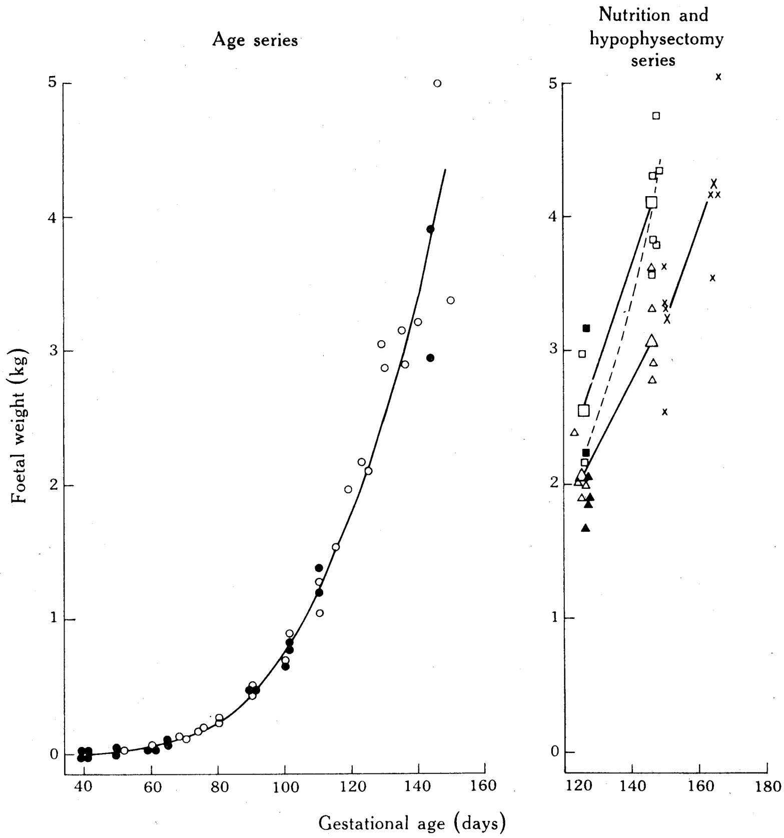

Fig. 1. Weight of foetal lambs at various stages of gestation. Age series: ○ singles, ● twins. Nutrition series: Restricted, △ singles, ▲ twins; *ad libitum*, □ singles, ■ twins. Hypophysectomy series: × singles. In the hypophysectomy series lambs were collected by Caesarian section either at normal full term or when about 16 days post-term. The large symbols (□, △ and ×) denote means (singles only). The trend line shown in the age series (singles) was fitted by eye. The upper part of this line (broken line in the right-hand graph) is included for comparison.

cutaneous fat. Of the 10 older foetuses examined five had no dissectible subcutaneous fat, indicating that the absolute weight of this depot declines in late pregnancy (13/13 *v.* 5/10, $P = 0\cdot008$ by Fisher's exact test). A decline relative to foetal weight is clearly apparent from 100 days (Fig. 3) (coefficient of correlation between relative weight and age of singles and twins beyond 100 days $= 0\cdot78$, $P < 0\cdot001$). The distribution of twins at various gestation ages was too uneven for a valid comparison with singles, but twins seemed to be as well-endowed with subcutaneous fat, relative to body weight, as singles.

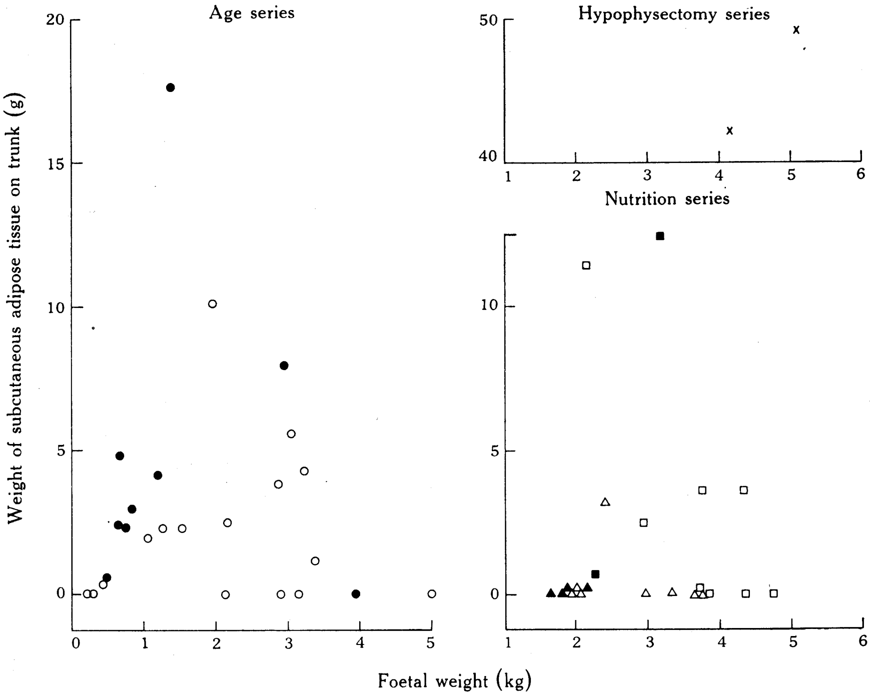

Fig. 2. Weight of subcutaneous adipose tissue on the trunk related to foetal weight. Symbols are as in Fig. 1. Note the large amount of this tissue in the two hypophysectomized foetuses that were dissected.

In the *nutrition series* there were clearly more foetuses with significant subcutaneous fat in the *ad libitum* group than in the restricted group (Fig. 3) (6/10 *v.* 1/12 respectively, $P = 0\cdot015$). This difference cannot be explained simply in terms of the effect on foetal weight; indeed, restricted foetuses of less than 3·5 kg clearly contained less subcutaneous fat than age series foetuses of the same weight and age (Fig. 2). In the *ad libitum* foetuses, on the other hand, this depot tended to be heavier than in age series foetuses of the same weight. All four *ad libitum* foetuses had dissectible amounts of subcutaneous fat (0·7–12·5 g) at 125–126 days but only two of the six had any (3·6 and 3·7 g) at 146–148 days ($P < 0\cdot05$ for singles, Table 2).

All *hypophysectomized* foetuses had an extensive covering of subcutaneous adipose tissue between 1 and 5 mm thick. The only two that were dissected had 42 and 49 g respectively, compared with the largest amount of 17·5 g (at 110 days) seen in any lambs of the other series.

Perirenal–abdominal depot

In the *age series* no dissectible adipose tissue was found in the perirenal–abdominal region in foetuses younger than 70 days, i.e. in foetuses of less than 150 g, but in older foetuses the amount of perirenal–abdominal fat increased with age to about 30 g near full term. Beyond 70 days the weight of the depot was clearly related to body weight (Fig. 4), although in foetuses of 3 kg or more variability was high. This variability was not readily explained in terms of varying gestation age, but there were too few animals for an adequate analysis of this aspect. The depot weight in twins was similar to that in singles of the same body weight (Fig. 4) but there were insufficient animals, especially near term, for an adequate examination of this point.

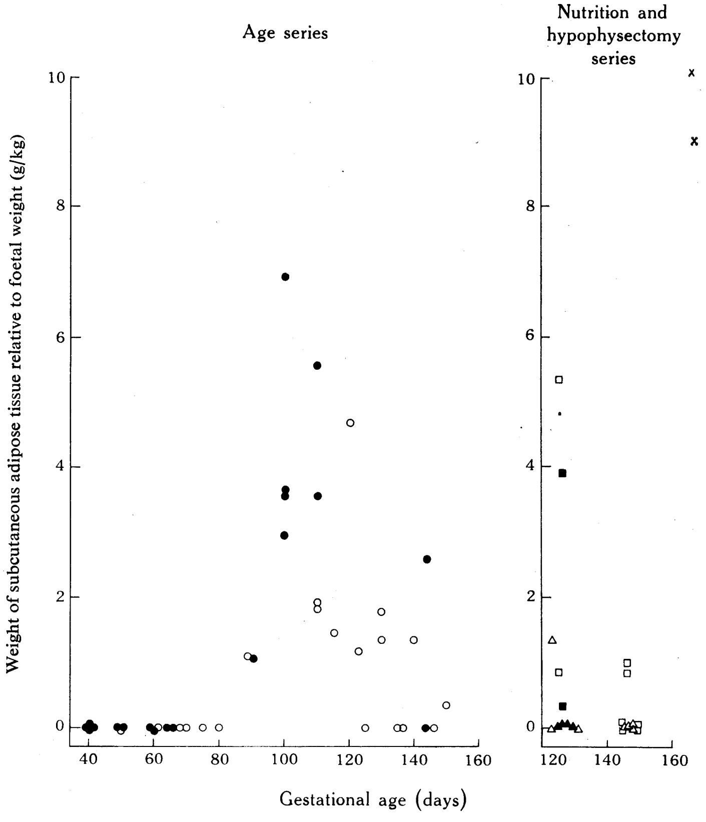

Fig. 3. Weight of subcutaneous adipose tissue per unit of foetal weight related to gestational age. Symbols are as in Fig. 1.

The size of the depot relative to body size (gram per kilogram of foetal weight) increased rapidly from zero at about day 70 to between 4 and 8 g/kg by day 110 (Fig. 4). Subsequent changes were highly variable but in contrast to the subcutaneous depot there was no clear decline in the relative size of the perirenal–abdominal depot during the last weeks of pregnancy. However, regression analysis reveals a non-significant trend for growth of this depot beyond 120 days to be lower than that from 65 to 120 days (regression coefficients $-0 \cdot 006 \pm 0 \cdot 073$ and $0 \cdot 100 \pm 0 \cdot 023$ g/kg per day respectively for single lambs).

In the *nutrition series* the mean weights of the depot relative to body weight (gram per kilogram) were higher in the *ad libitum* than in the restricted group (Table 2, Fig. 4), though the difference was significant only at 123–127 days ($P < 0 \cdot 05$ for singles; $P < 0 \cdot 01$ for twins). However, variability was again high, and depots that were small in relation to body weight (4 g/kg) were found in individuals from both treatment groups (Fig. 4).

In twins examined at 123–126 days the size of the depot in relation to body weight was less than in singles ($P < 0 \cdot 01$ for the restricted group; $P < 0 \cdot 05$ for the *ad libitum* group). There also appeared to be a trend for the relative size of the perirenal–abdominal depot to plateau or even decline in late pregnancy; the means at 146–148 days were less than the corresponding means at 123–127 days (Table 2, Fig. 4), although differences were not significant. There was also a trend for the depot weight in 146–148-day-old foetuses from ewes fed *ad libitum* to decline with increasing foetal weight (correlation coefficient $0 \cdot 77$, d.f. = 4, $P \simeq 0 \cdot 07$).

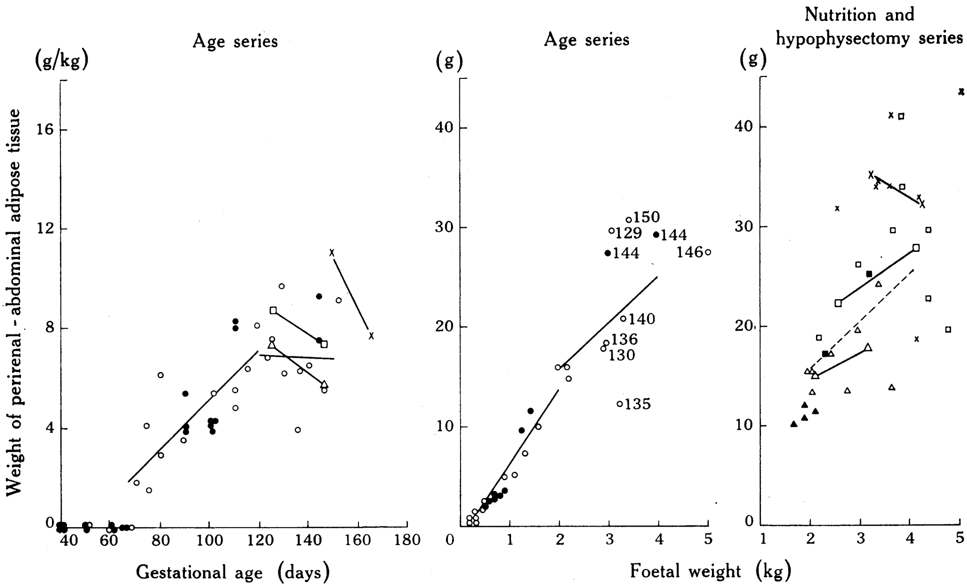

Fig. 4. Weight of perirenal–abdominal adipose tissue related to foetal weight in the three series (gestational ages of older foetuses indicated in age series); and weight of the same tissue expressed as weight per unit of foetus, related to gestation age. Trend lines for the age series foetuses aged 65–120 days (60 g–2 kg) and for older animals were fitted by regression analysis; the line for the older foetuses is included (broken line) with the nutrition series for comparison. Symbols are as in Fig. 1.

In seven of the eight *hypophysectomized* foetuses the perirenal–abdominal depot was heavier than in any of the age series foetuses of similar body weight (Fig. 4), and, as in the nutrition series, the mean weight of the depot relative to body weight was less in the older foetuses than in the younger ones ($7 \cdot 63 \pm 1 \cdot 11$ *v.* $11 \cdot 02 \pm 0 \cdot 55$ g/kg, $P < 0 \cdot 05$).

The weight of this depot in the age and nutrition series was significantly correlated with the weight of total extractable lipid in the foetus (Fig. 5) ($r = 0 \cdot 94$ and $0 \cdot 90$ respectively, $P < 0 \cdot 001$). However, the hypophysectomized foetuses contained more lipid than predicted from the linear regression relationships for normal lambs of the age and nutrition series.

Table 2. Nutrition series: weight of adipose tissue depots in relation to foetal weight

Values expressed are mean weights of adipose depots ± s.e.m. (gram per kilogram foetal weight). n.s., Not significant

Maternal nutritional treatment	Age (days)	No. of foetuses		Foetal weight (kg)		Perirenal–abdominal		Pericardial		Popliteal		Postorbital		Subcutaneous	
		Singles	Twins	Singles	Twins	Singles	Twins	Singles	Twins	Singles	Twins	Singles	Twins	Singles	Twins
Restricted	123–127	4	4	2·09 ±0·10	1·87 ±0·08	7·32 ±0·29	5·94 ±0·19	0·98 ±0·19	0·40 ±0·05	0·18 ±0·30	0·17 ±0·02	0·70 ±0·15	0·85 ±0·11	0·33 ±0·33	0·30 ±0·17
	146	4	0	3·15 ±0·19	—	5·67 ±0·81	—	0·74 ±0·05	—	0·11 ±0·10	—	0·59 ±0·04	—	0·0	—
Ad libitum	125	2	2	2·55 ±0·41	2·70 ±0·46	8·77 ±0·05	7·86 ±0·17	0·61 ±0·17	0·67 ±0·03	0·24 ±0·10	0·30 ±0·02	0·80 ±0·18	0·68 ±0·00	3·08 ±2·25	2·13 ±1·82
	146–148	6	0	4·11 ±0·18	—	7·36 ±1·00	—	0·71 ±0·08	—	0·11 ±0·02	—	0·58 ±0·06		0·30 ±0·19	—

Variable examined	Group in which test made	Statistical comparisons between pairs of means (t-tests) *P* values for various comparisons in each of the six parameters above					
Nutrition	*c.* 125-day-old singles	n.s.	< 0·05	n.s.	n.s.	n.s.	n.s.
(*ad lib. v.*	*c.* 125-day-old twins	*c.* 0·05	< 0·01	< 0·05	< 0·02	n.s.	n.s.
restricted)	*c.* 146-day-old singles	< 0·01	n.s.	n.s.	n.s.	n.s.	n.s.
Age (123–127 *v.*	Restricted singles	< 0·01	n.s. (< 0·1)	n.s.	n.s. (< 0·1)	n.s.	n.s.
146–148 days)	*Ad lib.* singles	< 0·01	n.s.	n.s.	< 0·05	n.s.	< 0·05
Litter size	*c.* 125-day-old, restricted	n.s.	< 0·01	< 0·05	n.s.	n.s.	n.s.
(single *v.* twins)	*c.* 125-day-old, *ad lib.*	n.s.	< 0·05	n.s.	n.s.	n.s.	n.s.

Other depots

Dissectible adipose tissue first appeared at the pericardial, popliteal and postorbital sites between day 70 and day 80 in the *age series*. As with the larger perirenal–abdominal depot, the weights of these small depots were obviously related to foetal weight. Variability was extremely high and there was no clear relation between gestation age, beyond 80 days, and the size of the depots relative to foetal weight in the age series. However, regression analysis again indicated a non-significant trend for the relative growth of the postorbital and popliteal depots to decline beyond 120 days (for single lambs regression coefficients from 65 to 120 days were $0 \cdot 0103 \pm 0 \cdot 0063$ and $0 \cdot 00276 \pm 0 \cdot 00075$ g/kg per day respectively, and from 120 days to term they were $0 \cdot 0032 \pm 0 \cdot 0105$ and $0 \cdot 0007 \pm 0 \cdot 0012$ g/kg per day respectively).

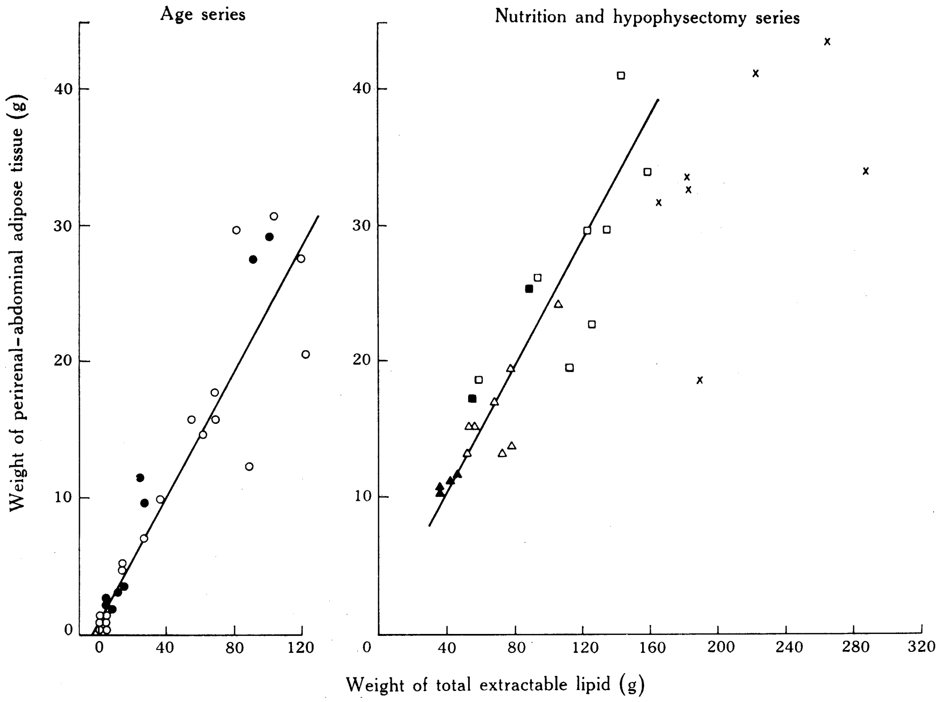

Fig. 5. Weight of perirenal–abdominal adipose tissue related to the total weight of chloroform-extractable lipid in the foetus. Trend lines for the age and nutrition series were fitted by regression analysis. Symbols are as in Fig. 1.

In addition the mean weights of the popliteal and postorbital depots relative to foetal weight in the *nutrition series* indicated a tendency for the weight to decline with advancing foetal age (Table 2); this was significant only with the popliteal depot in restricted single foetuses. The pericardial and popliteal depots were also significantly heavier, relative to body weight, in *ad libitum* twins than in restricted twins ($P < 0 \cdot 05$ and $P < 0 \cdot 02$ respectively), but there were no significant corresponding differences in single foetuses (Table 2).

In the two *hypophysectomized* foetuses in which these sites were examined the depot sizes were consistent with those in the other series.

Lipid Content of Dissected Adipose Tissue

The level of chloroform-extractable lipid in the dissected adipose tissue of the *age series* (all dissected depots pooled prior to extraction) was obviously related to foetal weight and age (Fig. 6) with values rising from 10% around 80 days, when the foetus weighed 0·4 kg, to 40 and 50% by 120 days, or a foetal weight of 2 kg, beyond which no further increase was apparent. The tendency for the curve to plateau during the last weeks of gestation was significant as shown by comparison of regression coefficients relating lipid content to foetal age below and above 120 days ($0 \cdot 859 \pm 0 \cdot 209$ *v.* $-0 \cdot 072 \pm 0 \cdot 169$% per day respectively for single lambs, $P < 0 \cdot 01$). This tendency was even more marked when the percentage lipid content was related to foetal weight below and above 2 kg ($23 \cdot 3 \pm 3 \cdot 8$ *v.* $-1 \cdot 8 \pm 1 \cdot 5$% per kilogram respectively, $P < 0 \cdot 001$) (Fig. 6).

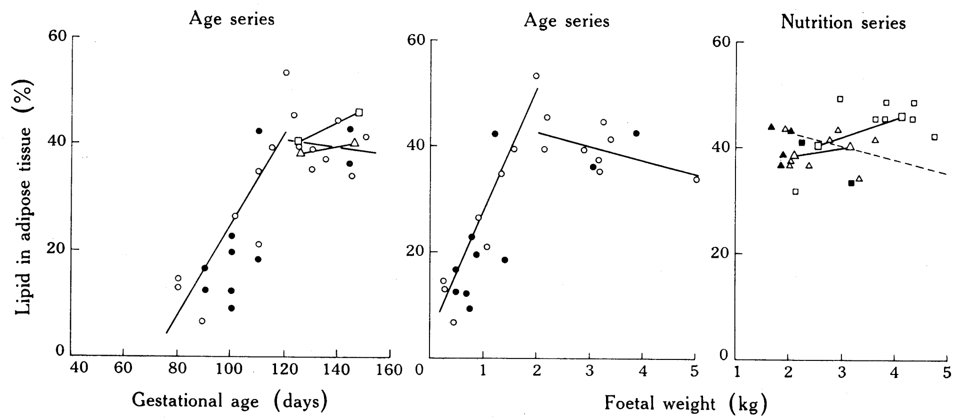

Fig. 6. Lipid content of dissected adipose tissue related to gestational age. Trend lines were calculated as in Fig. 4. Symbols are as in Fig. 1.

In the *nutrition series* the lipid content of the adipose tissue at 125–126 days was similar to that in the age series (*c.* 40%), and as in the age series any change in the content between 125–126 and 146–148 days was small (Fig. 6). At 146 days the content was significantly higher in the *ad libitum* singles than in the restricted singles ($P < 0 \cdot 05$) (Fig. 6, Table 3). There were no obvious differences between singles and twins.

Only two *hypophysectomized* foetuses (165 and 166 days) were examined, and in these the lipid content of the subcutaneous adipose tissue was determined separately from that of the other depots; the subcutaneous tissue contained 50 and 51% lipid and the other depots 31 and 33% respectively.

Total Lipid Content of Body

In the *age series* the percentage content of chloroform-extractable lipid in the whole body, adipose tissue included, was about 0·5% in 40–50-day-old foetuses weighing 10–20 g (Fig. 7). The subsequent lipid content began to increase, in a linear fashion, well before adipose tissue appeared, and reached a mean of 120 g at 4 kg body weight (3·0% at 145 days). Variability was high, but again there was a non-significant trend for the regression coefficient relating relative lipid content to

Table 3. Nutrition series: mean lipid content of various tissues

n.s., Not significant

Maternal nutritional treatment	Age (days)	No. of foetuses: Singles	No. of foetuses: Twins	Percentage extractable lipid (wet weight basis) in: Adipose tissue: Singles	Adipose tissue: Twins	Whole body: Singles	Whole body: Twins	Liver: Singles	Liver: Twins	Skeletal muscle: Singles	Skeletal muscle: Twins
Restricted	123–127	4	4	38·6±1·7	40·6±1·9	2·74±0·05	2·16±0·13	2·02±0·27	1·40±0·08	1·97±0·34	1·51±0·09
	146	4	0	40·3±2·0	—	2·60±0·16	—	1·58±0·09		1·25±0·12	
Ad libitum	125	2	2	40·5±8·6	37·3±3·5	2·96±0·19	2·66±0·14	1·52±0·16	1·66±0·26	1·69±0·11	1·61±0·20
	146–148	6	0	46·0±1·0	—	3·27±0·27	—	1·39±0·08		1·31±0·05	

Variable examined	Group in which test made	Statistical comparisons between pairs of means (*t*-tests). *P* values for various comparisons in each of the four parameters above: Adipose tissue	Whole body	Liver	Skeletal muscle
Nutrition	*c.* 125-day-old singles	n.s.	n.s.	n.s.	n.s.
(*ad lib. v.*	*c.* 125-day-old twins	n.s.	<0·05[A]	n.s.	n.s.
restricted)	*c.* 146-day-old singles	<0·05	<0·05[A]	n.s.	n.s.
Age (123–127 *v.*	Restricted singles	n.s.	n.s.	n.s.	n.s. (0·1)
146–148 days)	*Ad lib.* singles	n.s.	n.s.	n.s.	<0·01
Litter size	*c.* 125-day-old, restricted	n.s.	<0·01	n.s. (<0·1)	n.s.
(single *v.* twins)	*c.* 125-day-old, *ad lib.*	n.s.	n.s.	n.s.	n.s.

[A] One tailed *t*-test since differences in direction are expected from earlier studies.

age in singles to decline beyond 120 days ($0 \cdot 315 \pm 0 \cdot 085$ *v.* $0 \cdot 139 \pm 0 \cdot 183$ g/kg per day for 60–120- and 120–145-day-old foetuses respectively. There was no evidence that twins differed from singles in this respect.

In the *nutrition series*, as in the age series, any increase beyond 120 days was small. The percentage was higher in the *ad libitum* group than in the restricted foetuses (Fig. 7, Table 3) ($P \simeq 0 \cdot 05$ for singles at 146–148 days and for twins at 123–127 days). Twins contained less lipid per unit of body weight than singles ($P < 0 \cdot 01$ at 123–127 days).

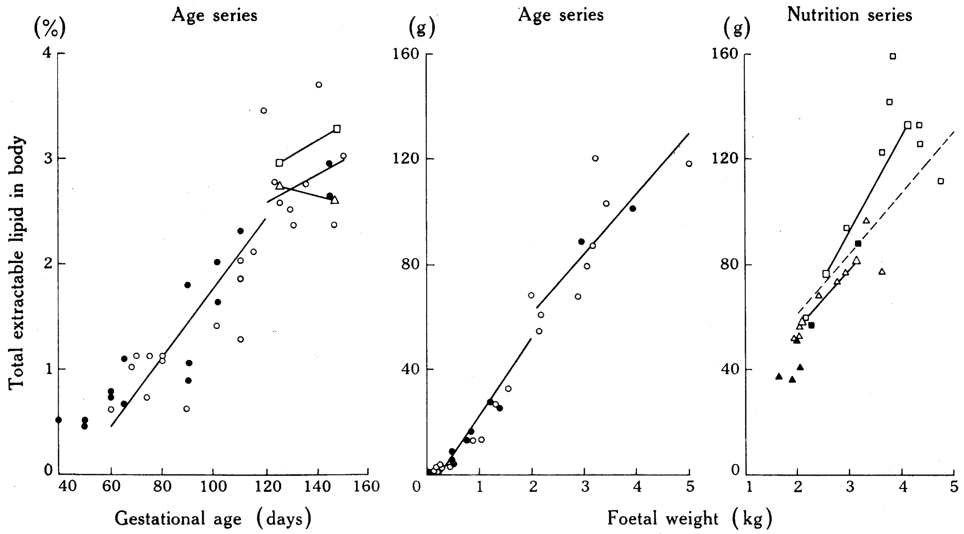

Fig. 7. Weight of chloroform-extractable lipid in the whole foetus related to gestational age. Trend lines were calculated as in Fig. 4. Symbols are as in Fig. 1.

Hypophysectomized foetuses contained amounts of extractable lipid that ranged from $4 \cdot 4$ to $7 \cdot 5\%$ of body weight (average $5 \cdot 8\%$ at 148–150 days, $5 \cdot 4\%$ at 164–168 days; difference not significant); all percentages were higher than those in any other foetus.

Lipid Content of Liver and Muscle

In the *age series* the lipid contents of liver and skeletal muscle freed of visible adipose tissue were examined in the second year of the study only; both declined significantly with advancing foetal age ($P < 0 \cdot 01$ in regression analysis). At 60–70 days the liver contained *c.* 3% lipid and muscle *c.* 5%; near term the values were $1 \cdot 6\%$ and $1 \cdot 5\%$ respectively. Over this period liver increased from 10 g to more than 100 g while the weight of the sampled muscles increased from $0 \cdot 5$ g to more than 30 g. There were too few data to show any trends during the last few weeks of foetal life.

In the *nutrition series* there were no clear differences in the lipid content of liver between the restricted and *ad libitum* foetuses; in both groups the content of the older foetuses tended to be less than that of the younger foetuses, but differences were not significant (Table 3). Likewise there were no consistent differences in the lipid content of muscle, between the two nutritional groups, but the means of the older animals were less than those of the younger animals ($P < 0 \cdot 1$ and $P < 0 \cdot 01$ for restricted and *ad libitum* respectively) (Table 3), indicating that the lipid content of at least some non-adipose tissues declines during the last weeks of gestation.

In the two *hypophysectomized* foetuses examined at 165 and 166 days the liver contained 2·2 and 2·6% lipid and the muscle sample 2·5 and 2·1% respectively; these values are higher than any in near-term lambs in the other series.

Discussion

The present study indicates that Merino foetuses begin to accumulate chloroform-extractable lipid at about day 50, prior to the appearance of dissectible adipose tissue. From about day 70 there is a very rapid growth, relative to body weight, of the non-subcutaneous adipose depots ('brown fat'), and a parallel increase in their lipid content, lasting until days 110–120, so it may be largely during this period of fat organogenesis that the ultimate amount of brown fat in the individual animal is determined. Subcutaneous adipose tissue ('white fat') appears between days 80 and 90, but its development follows a very variable pattern, and there appears to be a marked involution with advancing gestation, which is consistent with the failure of the author and colleagues (Gemmell *et al.* 1972; Alexander and Bell 1975) and Liggins and Kennedy (1968) to find any more than traces of subcutaneous fat in normal full-term lambs.

In newborn rabbits white fat has been shown to be more readily depleted than brown fat during fasting at thermoneutrality (Heim and Kellermayer 1967; Hardman *et al.* 1969); hence the disappearance of white fat in sheep foetuses may reflect a sub-optimal supply of nutrients during the phase of very rapid foetal growth. This possibility is consistent with the adverse effects of maternal nutritional restriction on the white fat content of foetuses (Figs 2 and 3). Also, in view of the relative absence of subcutaneous fat from the 146-day-old foetuses of the well-fed group it seems that the nutrient supply to the foetus could be limited at the placental level rather than by the concentration of substrates in the maternal circulation. Other trends in the data, some of which require confirmation, are also consistent with the hypothesis that the nutrient supply of the rapidly growing, near-term sheep foetus is becoming inadequate. Thus, in the age and nutrition series the growth of the brown fat depots relative to body weight tended to plateau or even decline during the last few weeks of gestation; hypophysectomized foetuses showed a significant decline in the proportion of perirenal–abdominal fat in the body with advancing age; and the proportion of lipid in skeletal muscle of foetuses in the nutrition series likewise declined. Also, maternal undernutrition was shown to reduce the size of some of the brown fat depots, the lipid content of the body as a whole, and the adipose tissue near term, independently of its effects on foetal weight. Although there were no significant trends in the total chloroform-extractable lipid content of near-term foetuses in the present study, Rattray *et al.* (1974) found that this tended to plateau after 125 days.

The hypothesis that foetal nutrition near term is suboptimal is consistent with the well-established finding that the nutritional requirements of the ewe increase during the last third of pregnancy, and that maternal blood glucose level tends to decline as ovine pregnancy advances (Reid and Hinks 1962). Maternal glucose is the major energy source of foetal lambs (Burd *et al.* 1975), and the degree of foetal adiposity may reflect the abundance of maternal blood glucose. Nevertheless, it is remarkable that the white fat should begin to involute as early as 110–120 days. Perhaps undernutrition is the normal physiological state of the maturing foetus; indeed advancing undernourishment could play some role in triggering the process of foetal maturation and parturition (Alexander 1976). Foetal undernutrition could also account for at least some of the normal variation in the rate of foetal growth, such as that normally encountered between twins and singles in sheep (Starke *et al.* 1958). Rattray *et al.* (1974) observed a tendency for growth of twin foetuses to slow in late gestation, especially where maternal nutrition was restricted. In the present study few twins were encountered near term, but those in the nutrition series tended to have smaller fat depots relative to body weight and less lipid in the body than singles.

Other explanations for the depletion of white fat in foetuses are also possible. For example, there are high levels of thyroid hormones in sheep foetuses during the last third of pregnancy (Hoskins and Thorburn 1971) which could affect foetal metabolic rate and fat deposition. An endocrinological explanation is supported by Liggins and Kennedy's (1968) observation, confirmed here, that hypophysectomized sheep foetuses are comparatively obese. Hypophysectomized sheep foetuses tend to have a somewhat higher proportion of brown fat, very much larger subcutaneous depots of white fat, and more total body chloroform-extractable lipids than normal lambs, yet their body growth appears to have been only slightly slower than normal. The obesity could result from a decrease of protein synthetic capacity due to a deficiency of growth hormone or derivatives (Gaebler *et al.* 1951), with a resulting increase in availability of nutrients for storage as fat, or from a more direct effect on the balance between lipolysis and lipogenesis, in favour of the latter (Wiel 1965). Also thyroidectomized foetuses have a very low metabolic rate (Hopkins and Thorburn 1972) so hypophysectomy, which results in depressed thyroid function, could facilitate the accumulation of lipid. There is, however, some doubt about whether the obesity that follows hypophysectomy in rats and dogs is due to the absence of the pituitary or due to hypothalamic damage (Smith 1930).

It has been pointed out previously that newborn Merino lambs contain about 1·5% of dissectible adipose tissue, and that in a 4-kg lamb this represents about 24 g of extractable lipid or only about 30% of the 80 g of utilizable lipid (Alexander 1962), so that much of the utilizable lipid appears to be stored at sites outside the dissectible adipose tissue (Alexander and Bell 1975). The liver could account for only 2 g. It is not clear how much of the lipid in muscle is available for metabolism, or whether thigh muscle is representative of all skeletal muscle; Wallace (1948) reported values of 2·5–3·0% lipid in skeletal muscle of crossbred Suffolk lambs. However, if thigh muscle of Merino lambs is representative, a 4-kg lamb would contain some 20 g of lipid in this tissue, representing a significant part of the lipid that is unaccounted for.

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